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Elastic deformation of compact bone

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Comparative microstrain measurements of the elastic deformation of tibia compact bone sections taken from 1-, 3- and 12-month-old rabbits, revealed an increase in the elastic modulus from 15.1 to 27.6 GN m^{-2} with age. This result was correlated with observations of the porosity and hydroxyapatite percentages of the compact bone and the implications for a collagen/hydroxyapatite composite were examined.

1. Introduction

During the last decade, there have been a number of contributions to an interpretation of the elastic modulus of compact bone in terms of its collagen and hydroxyapatite constituents. The original concept of bone acting as a hydroxyapatite reinforced composite was suggested by Currey [1]. Later work by Bonfield and Li [2-4] on bovine compact bone, demonstrated the importance of distinguishing the elastic modulus from the non-elastic components of deformation. These results were used to evaluate quantitatively a composite model of modulus based on the linear rule of mixtures, given by

$$E_b = E_h V_h + E_c V_c \quad (1)$$

where E_b, E_h and E_c are the Young moduli of bone, hydroxyapatite and collagen and V_h and V_c are the volume fractions of hydroxyapatite and collagen, respectively. This simple model gave reasonable agreement between the calculated modulus values and the experimental data then available for hydroxyapatite and collagen (E_h = 63.5 GN m^{-2}, E_c = 1.3 GN m^{-2}). However, Katz [5] subsequently measured the modulus of mineral and synthetic hydroxyapatite ultrasonically and obtained a significantly higher value (E_h = 114 GN m^{-2}), which indicated that Equation 1 did not satisfactorily account for the modulus of bone, although his comprehensive review of other two-phase models failed to distinguish an acceptable alternative. An additional factor of importance was highlighted by Currey [6], who found a sensitive dependence of the elastic modulus of rabbit metatarsals, on ash content, which on the basis of Cox's general composite treatment [7], he attributed [8] to changes in the length/diameter ratio of the hydroxyapatite crystals.

The critical test of the suitability of a particular model for the elastic modulus of bone appears to be provided by the dependence of modulus on the volume fraction of hydroxyapatite. Unfortunately, to achieve a large variation in the hydroxyapatite volume fraction, it is necessary to include values for different materials (e.g. dentine, enamel), an exercise which is inevitably complicated by other structural variations. An alternative approach is to study sections of a given bone, but with the sections taken from animals of different age in order to obtain a small variation in hydroxyapatite volume fraction. As any resultant changes in modulus are also likely to be small, this approach demands a precise measurement of the elastic and non-elastic components of deformation, and control of strain-rate. In addition, any associated structural changes, e.g. variations in the porosity content arising from the lacunae and vascular spaces, should be accounted for. In this paper, some preliminary results on the elastic deformation of rabbit compact bone tibia sections, taken from 1-, 3- and 12-month-old animals, are presented which demonstrate the possibilities of such an approach. It is shown that the elastic modulus of compact bone increases significantly with age and is dependent on the hydroxyapatite volume fraction and modulus and the porosity content.

2. Experimental procedure

2.1. Specimen preparation and testing

Tensile specimens, as shown in Fig. 1, were

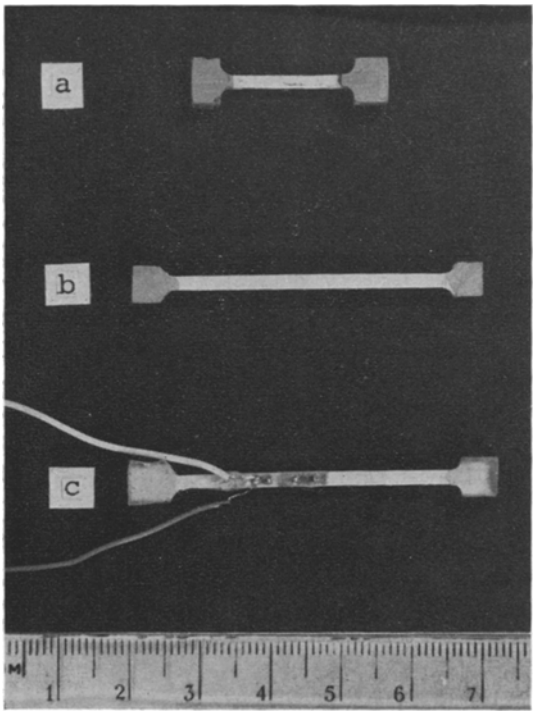

Figure 1 Tibia compact bone tensile specimens (longitudinal sections) (a) 1 month (b) 3 month (c) 12 month.

prepared from tibia sections taken from New Zealand White rabbits of age 1, 3, and 12 months respectively. The bone was slit into three sections, the ends of which were set in five-minute-setting epoxy resin and the gauge length obtained by milling of the slit edges. The specimen shoulders were milled to a radius to conform with the contours of the specimen grips. No material was removed from the periosteal surface (except for slight roughening for gauge adhesion) but the irregularities on the endosteal surface were removed with a small file, to enable an accurate measurement of area, and to provide a flat surface for the strain gauge. At all stages in this procedure (except for the resin mounting) the bone was immersed in or kept moist with Ringers solution and during actual testing a plastic envelope was placed around the specimen.

For an accurate measurement of deformation, it is essential to take the strain readings from the gauge length of the specimen. This was achieved by attaching Micro-Measurements EA series epoxy backed foil resistance strain gauges ($3.8 \times 10^{-4} \times 7.9 \times 10^{-4}$m) to the specimen. The gauges were connected to a direct reading strain indicator (Vishay-Ellis 20A), which consists of a Wheatstone bridge and a digital voltmeter, and gives a strain sensitivity of 1×10^{-6}. One gauge was mounted on each side of the specimen and the pair wired in series to compensate for any specimen bending. In addition, a control pair of gauges was mounted on an adjacent piece of bone, to constitute the fourth arm of the bridge and compensate any temperature variation.

The test specimen was secured rigidly in the specimen grips, but mounted in an Instron testing machine by a flexible ball joint coupling which allowed accurate alignment. Prior to testing, the specimen was equilibriated at room temperature. It was then deformed at a constant strain-rate between 1 and 3×10^{-4} sec^{-1}.

For any strain gauge system, it is desirable to demonstrate that the strain gauge output represents the deformation of the bone alone and is equivalent to some given direct reading of strain. This calibration was achieved by mounting both the strain gauges and a Tuckerman optical strain gauge [2] (with mechanical contact, measurement over 2.5×10^{-2} m and a strain sensitivity of 2×10^{-6}) on a bovine femur specimen, which as shown in Fig. 2, gave agreement within 10% between the two techniques.

2.2. Structural characterization

2.2.1. Histological sectioning

The bone was decalcified in 1% nitric acid and embedded in 20% celloidin solution. The blocks formed were double embedded in paraffin wax and sections (6 to 10 μm in thickness) for optical microscopy were cut with a base-sledge microtome. The sections were stained either with Mallory's connective tissue stain, which exhibits the lamellar structure or with haematoxylin and eosin, which shows the general structure and particularly the cement lines.

The area of the section occupied by vascular spaces and cell lacunae was measured with a planimeter and measuring eyepiece. Assuming that the same percentage area is occupied by lacunae and spaces in any negligibly thick section, the percentage area also represents the percentage volume of space in the bone.

2.2.2. Ashing

Bone sections were dried for 24 h at 45° C, allowed to equilibriate for 1 h in contact with the atmosphere and accurately weighed. The sections were then heated in a crucible over a hot bunsen flame, allowed to equilibriate for 1 h at room

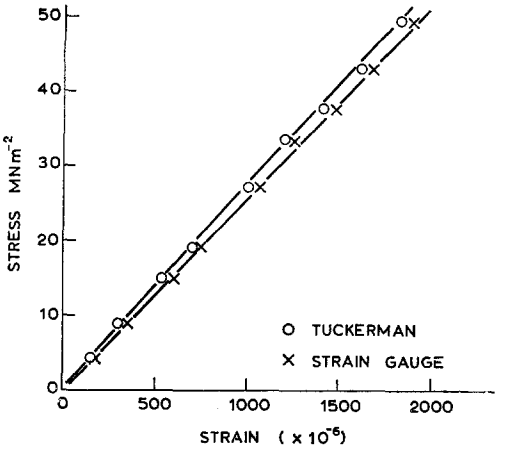

Figure 2 A comparison of strain gauge and Tuckerman gauge measurements from a longitudinal bovine femur section (~ 36 month), deformed in tension at 2×10^{-4} sec^{-1}.

temperature and reweighed. This procedure was repeated until a constant weight was achieved. The residual ash content was calculated as a percentage of the initial "dry" weight and taken as the hydroxyapatite weight percentage.

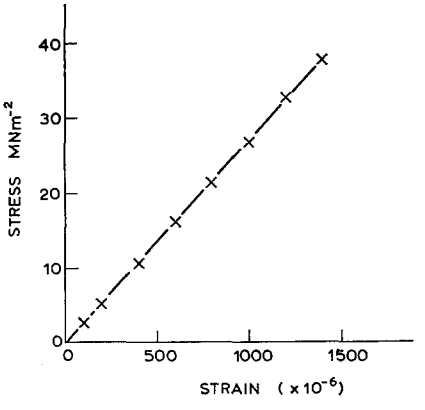

Figure 3 Stress-strain behaviour of a longitudinal 12-month rabbit tibia compact bone section, deformed in tension at 2×10^{-4} sec^{-1}.

3. Results

3.1. Modulus measurements

A typical stress-strain measurement for a 12 month specimen is shown in Fig. 3. The features of the deformation were the linear slope to a relatively high stress level and the absence of hysterisis. It was found that the modulus obtained from the linear slope, increased initially with an increase in strain-rate, but achieved an approximately constant value above ~ 1×10^{-4} sec^{-1} (Fig. 4). Hence, the measurements of modulus were made at a strain-rate of 1 to 3×10^{-4} sec^{-1}. The average value of modulus obtained in thirteen tests was 27.6 GN m^{-2}, with a range from 27.1 to 28.3 GN m^{-2}.

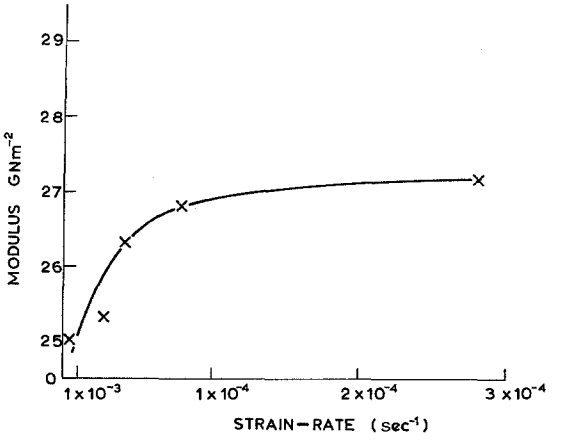

Figure 4 The effect of strain-rate on the elastic modulus of a 12-month rabbit tibia section, deformed in tension.

Similar types of stress-strain curves were obtained for the 3- and 1-month specimens, but the average modulus values were different at 23.6 GN m^{-2} (five tests, range 22.5 to 24.2 GN m^{-2}) and 15.1 GN m^{-2} (five tests, range 14.6 to 16.0 GN m^{-2}), respectively.

3.2. Porosity and hydroxyapatite percentages

Typical optical micrographs from histological sections from the 1-, 3- and 12-month bone are shown in Fig. 5. Measurements were made of the area occupied by cell lacunae and vascular spaces to assess the total porosity. It can be seen that the porosity decreases significantly from the 1 to the 3-month section, but is approximately the same for the 3- and 12-month sections. The average values obtained for eight sections were ~ 27% (1 month) (range 21 to 30%) and ~ 10% (3 and 12 month) (range 6 to 11%).

The average ash contents obtained for the 1-, 3- and 12-month sections (from 15 to 26 specimens) were 65.6 (range 61 to 68%), 65.8 (range 61 to 68%) and 70.0% (range 66 to 74%) respectively. Taking the density of hydroxyapatite and collagen as 3.17×10^3 kg m^{-3} and

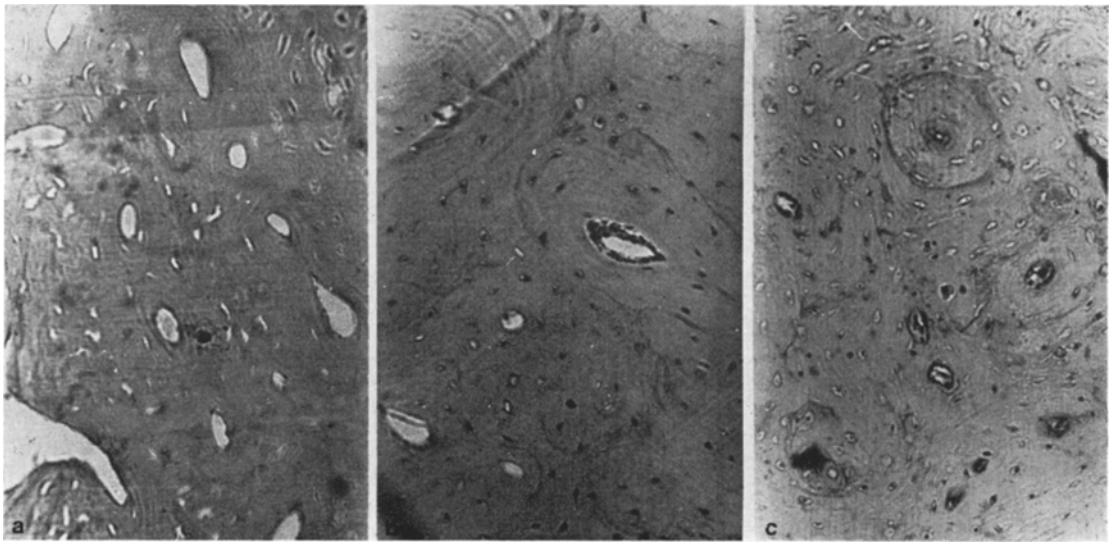

Figure 5 A comparison of the microstructure of (a) 1 month, (b) 3 month and (c) 12-month rabbit mid-shaft tibia histological sections (×160).

1.33×10^3 kg m^{-3}, the average hydroxyapatite volume fractions are 0.45 (1 and 3 month) and 0.50 (12 month).

4. Discussion

The elastic modulus measured for the 12-month tibia compact bone sections was 27.6 GN m^{-2}, a value which is in reasonable agreement with the modulus (26.5 GN m^{-2}) measured in tension [2] on bovine tibia sections at a comparable strain-rate. Both these results are significantly higher than the modulus of 19.0 GN m^{-2} obtained in compression [9] on bovine femur sections at a strain-rate of 10^{-3} sec^{-1}, and also differ from measurements in bending [6] on rabbit metatarsals (E = 7.8 to 15 GN m^{-2}). An important feature of the present results is that the modulus remained approximately constant above a critical strain-rate ($\sim 1 \times 10^{-4}$ sec^{-1}), which suggests that the anelastic strain contribution was negligible. This conclusion appears confirmed by ultrasonic measurements of bone modulus [10], which gave comparable values to the current "static" values. These results are in contrast to the marked dependence of modulus on strain-rate, measured in compression [9], from 10^{-3} to 3×10^2 sec^{-1}.

The elastic modulus measured for the 3-month sections was 23.6 GN m^{-2}, and for the 1-month sections was 15.1 GN m^{-2}. There are no other comparable measurements on these conditions. The importance of these results is in demonstrating that, for a given bone, tested under identical conditions, the elastic modulus increases significantly with the age of the bone. The three values obtained demonstrate the dependence of elastic modulus on both the hydroxyapatite and porosity content of compact bone. These effects may be considered separately, as the decrease in porosity from 1 to 3 months occurred at an approximately constant hydroxyapatite percentage, while the increase in hydroxyapatite percentage from 3 to 12 months occurred at a constant porosity level. We have:

(a) effect of a decrease in porosity.

With only two values of porosity, it is not possible to establish the dependence of modulus on porosity. Consequently, assuming for a first approximation that MacKenzie's equation [11], which relates the absolute modulus E_0 (i.e. with no porosity) to the measured modulus (E) and the porosity volume (p) is applicable, we may write, for compact bone

$$E_b = E_{b_0}(1 - 1.9p + 0.9p^2) \quad . \qquad (2)$$

Substituting in Equation 2 for $E_{b_{1\ month}}$, $p_{1\ month}$, $E_{b_{3\ month}}$ and $p_{3\ month}$, we obtain

(1) for the 1-month specimens E_{b_0} = 27.4 GN m^{-2}

(2) for the 3-month specimens E_{b_0} = 28.8 GN m^{-2}.

Hence, a correction for porosity for the 1- and

3-month results gives values of the absolute modulus which are in reasonable agreement.

(b) Effect of an increase in hydroxyapatite percentage.

Rewriting Equation 1 in terms of the absolute bone modulus, (E_{b_0}) we have

$$E_{b_0} = E_h V_h + E_c V_c \ . \quad (3)$$

Substituting in Equation 3 E_{b_0} and V_h for the 3-month results, and assuming $E_c V_c \sim 0.6$ GN m^{-2} [3] we obtain,

(1) for the 3 month specimens $E_h = 62.7$ GN m^{-2}.

Then substituting in Equations 2 and 3 for the 12-month results, we have

(2) for the 12-month specimens

$$(E_{b_0} = 33.5 \text{ GN m}^{-2})$$
$$E_h = 65.8 \text{ GN m}^{-2}.$$

Therefore, the hydroxyapatite modulus derived for the 3- and the 12-month bone is reasonably similar. Consequently, if the analysis was reworked from this standpoint, i.e. that E_h is constant, the increase in the absolute modulus of tibia compact bone from 3 to 12 months would be predicted on the basis simply of the increase in hydroxyapatite content.

TABLE I The variation of the measured bone modulus (E_b), the absolute bone modulus (E_{b_o}) and the hydroxyapatite modulus (E_h) with the age, porosity content and hydroxyapatite volume fraction (V_f) of longitudinal rabbit tibia sections.

Age (month)	Porosity (%)	V_f	E_b GN m^{-2}	E_{b_o} GN m^{-2}	E_h GN m^{-2}
1	27	0.45	15.1	27.4	59.6
3	10	0.45	23.6	28.8	62.7
12	10	0.50	27.6	33.5	65.8

However, if the three values of absolute modulus (E_{b_0}) for the 1, 3- and 12-month sections are considered (Table I), there is a significant increase in E_{b_0} with age that is not accounted for entirely by the increase in the hydroxyapatite volume fraction. This change in E_{b_0} may be attributed to the accompanying increase in the derived hydroxyapatite modulus (E_h) (Table I). If the 1-month section results are taken as a base, then the increase in hydroxyapatite volume fraction (V_h) and the increase in hydroxyapatite modulus (E_h) each contribute $\sim 50\%$ to the increase in the absolute bone modulus (E_{b_0}) noted for the 12-month sections.

The reasons for the increase in the hydroxyapatite modulus with age remain to be resolved, but could result from an increase in the crystalline/amorphous hydroxyapatite ratio [12] and/or the hydroxyapatite orientation and aspect ratio [8] (although the magnitude of the increase in absolute modulus is less than in Curreys, findings [6] on rabbit metatarsals). Clearly further data are required to critically test the hydroxyapatite and porosity dependence and experiments are in progress on femur, tibia, scapula and metatarsal sections.

The value of hydroxyapatite modulus derived is similar to that obtained from measurements [5] on enamel (98% hydroxyapatite) (77 to 84 GN m^{-2}). It differs significantly from the modulus measured [5] on powdered hydroxyapatite (114 GN m^{-2}), but the applicability of this particular value to rabbit tibia sections, in which approximately 30% of the hydroxyapatite is amorphous [12] has yet to be demonstrated.

5. Conclusions

1. The elastic modulus of rabbit tibia compact bone at a strain-rate of 10^{-4} sec^{-1} varied with age from 15.1 GN m^{-2} (1 month) to 23.6 GN m^{-2} (3 month) to 27.6 GN m^{-2} (12 month).
2. The increase in modulus with age was attributed to three factors: (a) a decrease in the total porosity content, (b) an increase in the hydroxyapatite volume fraction, and (c) an increase in the hydroxyapatite modulus.

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